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(54) **NOVEL INFECTIOUS BURSAL DISEASE VIRUS**

**NEUES VIRUS, VERANTWORTLICH FÜR DIE INFEKTIOSE ERKRANKUNG DER BURSA  
FABRICIUS**

**NOUVEAU VIRUS DE LA MALADIE INFECTIEUSE DE LA BOURSE DE FABRICIUS**

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**US-A- 4 530 831** **US-A- 4 824 668**

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91275k/

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## Description

### Technical Field:

This invention relates to the poultry industry, and in particular, infectious bursal disease, a known scourge of this industry. Specifically, a novel virus is identified, and methods of using this virus and information associated therewith are disclosed.

### Background Art:

Infectious bursal disease (IBD) has previously been identified as a significant economic drain in the poultry industry. This disease, which strikes chiefly at the chicken industry, is caused by virulent field viruses which cause a highly contagious, immunosuppressive disease condition. This condition, of course, exacerbates other infections in the chicken population. The disease is noted for its impact on young chickens, and is characterized by lesions in the lymphoid follicles of the bursa of Fabricius.

In U.S. Patent Application Serial Number 07/061,083, filed June 12, 1987, the inventor herein, and others, reported the development of two monoclonal antibodies sensitive to, and capable of neutralizing, all known viruses identified as inducing IBD. Indeed, that application, which is still pending, addresses the monoclonal antibodies, particularly those identified as R63 and B69, expressed by hybridoma cell lines deposited under ATCC HB-9437 and HB-9490, which continue to prove their value as neutralizing monoclonal antibodies, comprising a passive vaccine against known strains of viruses inducing IBD.

Nonetheless, recent history in the poultry industry, particularly that along the eastern coast of the United States, reflects an increasingly large number of reports of outbreaks of infectious bursal disease, which are not fully prevented by any of the known vaccines, including those prepared from the monoclonal antibodies discussed above. Due to the severe economic strain placed on the poultry industry by these uncontrolled outbreaks, a significant degree of investigation of the cause of the outbreaks, and the reason for the failure of known vaccines to prevent such outbreaks, has been undertaken. No fault has been detected in the preparation of the vaccines, or their administration. Nonetheless, unchecked outbreaks continue to occur.

Accordingly, it remains a persistent problem of the prior art to determine the cause of these outbreaks of infectious bursal disease which are resistant to any of the known vaccines, and determine a method of preventing further such outbreaks.

### Disclosure of the Invention

It has now been discovered that a major virus responsible for infectious bursal disease in poultry along the east coast of the USA is a newly identified, variant

strain with altered recognition sites, such that neither of the previously developed monoclonal antibodies are capable of neutralizing or binding the virus. However, these monoclonal antibodies do neutralize and react with all known IBD vaccines of the current art. Such monoclonal antibodies are described in J. General Virology, 66 (12) 1985, 2693-2702, in US-A-4 530 831 and in Biological Abstracts, 84, 15. 12. 87, Abstr. 120283.

The virus has been isolated in essentially pure form and can be identified by the failure of monoclonal antibody R63 and B69 to bind thereto, while another common non-neutralizing antibody as well as standard polyclonal antisera available from the USDA will bind thereto in positive fashion.

The new virus may be used in killed form as killed vaccines inducing antibodies resistant to the new virus, and may be used in attenuated form or otherwise genetically altered to prepare either a live or killed virus vaccine.

### Best Mode For Carrying Out The Invention

As noted above, the new virus, drawn from at least 116 novel isolates, taken from the east and Southeastern United States are not neutralized by any of the monoclonal antibodies previously developed and described. Thus, identification of the presence of the new virus cannot be achieved through normal measures. However, by a combination of negative and positive testing, the presence of the virus and isolation of the virus can be achieved.

In particular, the monoclonal antibody designated R63, which neutralizes all previously identified serotype one IBD virus strains and at least one serotype two gave negative results in an antigen capture-ELISA when reacted with the homogenized bursas drawn from chickens which yielded the twenty-three isolates. The same results were observed with MCA-B69, selective for the D78 virus strain and certain classic viruses of an earlier art, once thought to be the prevalent strain in the United States. At the same time, another MCA designated B29, expressed by a hybridoma cell line deposited at the ATCC under accession number MB 9746, pursuant to Budapest Treaty conditions, which does not neutralize the virus, nevertheless binds to it, as well as to all known existing virus vaccines. Additionally, the polyclonal IBDV antisera used as a standard, and available from the USDA's national veterinary services Laboratory in Ames, Iowa under designation ADU8701, binds, in the antigen capture ELISA, to the novel vaccine. Other non-neutralizing antibodies can be identified which bind to the virus, and can be directly produced as conventional monoclonal antibodies. The invention is not limited to any given positive test factor. Since the overall size of the virus, in comparison to any available neutralization site is quite large, there will be a large potential field of such positive test factors and polyclonal antisera.

Thus, the presence of the virus can currently be best determined by negative testing in an antigen capture-

ELISA for R63 and B69, and positive testing with either B29 or the polyclonal antisera. It should be noted, however, that morphological or symptomatic verification of the presence of an IBD virus, coupled with a failure of the R63 MCA to bind to an antigen sample, is clear evidence of the presence of the virus.

#### IDENTIFICATION OF THE VIRUS PRESENCE

To originally identify the presence of the new virus, 116 chicken populations were sampled, bursas were obtained from 116 distinct flocks on the Delmarva Peninsula and the Southeastern U.S. poultry rearing areas. Bursas from the 116 chicken populations were homogenized by placing one bursa in one ml of SGPA-EDTA buffer and grinding the mixture with a mortar and pestle until fluid-like consistency was obtained. This material was clarified by low speed centrifugation, and the supernatants were analyzed by an AC-ELISA.

In this assay, 96-well Immulon 1 plates (obtained from Dynatech, of Virginia) were coated with 0.1 ml of two ug/ml of protein A from *Staphylococcus aureus* in a coating buffer. After 18 hours at 4°C, the plates were dumped. 1/10 Dilutions of acid supernatants collected from hybridoma cultures secreting the R63 and B69 IBD virus specific MCAs were added in the phosphate buffered saline which contained TWEEN 20 and 2% non-fat dried powdered milk, in alternating fashion. After a 24 hours reaction at 4°C, the plates were tapped dry and blocked for 30 minutes at room temperature. After blocking, the plates were emptied and tapped dry. 0.1 ml of serial dilutions of each sample of the homogenized bursal suspensions were added to the coated plates, and after incubation, the plates were emptied, tapped dry and washed three times for three minutes with PBS-T. Then, each well received 0.1 ml of a biotin labelled R63 MCA conjugate, which was diluted in PBS-T + NFDm. After an hour of incubation, the plates were again emptied and washed. Subsequently, 0.1 ml of a streptavidin-horse-radish peroxidase conjugated was added to each well. After one hour of incubation the plates were again emptied and washed. This was followed by the addition of a TMB substrate. After a brief incubation period, the tests were read at 650 nm with the aid of an automated spectrophotometer. Thus, the biotinylated R63 MCA was used to signal for positive reactions between the virus and R63 and B69 wells, while a similar AC-ELISA was performed with a polyclonal anti-IBDV sera was used to signal the B29 catches. Alternatively, biotinylated B29 could be used to the same effect. Further, any form of labeling of R63, B69, B29, polyclonal may be used.

All 116 strains showed negative for reactivity with R63 and B69, but were highly positive for the B29 MCA, which combines in a non-neutralizing fashion.

As R63 is a neutralizing antibody for all previously identified IBD viruses, an assay employing only R63 as the positive non-neutralizing assay is adequate. The added use of B69 gives a higher confidence level, and

can be used to further define and separate IBDV strains of the prior art.

#### CONFIRMATION OF THE PURITY AND VIRULENCE OF THE VIRUS

Samples from five of the identified isolated strains, which virus is expressed by the deposits at the Institute Pasteur pursuant to Budapest Treaty conditions under accession numbers i.-792 and i.-793 were pooled, and reacted with the R63 MCA, and inoculated into SPF chickens. Five days after inoculation, these chickens, and non-inoculated chicken were necropsied. Those birds inoculated with the collected pool, referred to as NegaVac (NV) showed lesions consistent only with infectious bursal disease.

For certainty, antisera from the birds was taken at 11 days past inoculation, and was tested by indirect ELISA and showed serologic conversion to IBDV, but to no other related poultry diseases. Bursal samples from these birds were homogenized and passed a second time in the presence of R63 and B69 with identical results. In both passages, on a scale of 0-9, reactivity with the B29 MCA was at level 9, and reactivity with B69 and R63 was at level 0. Thus, a pure preparation of a previously unidentified virus, not related to any known vaccine at the R63 and B69 neutralization sites, prepared from virus or otherwise, was identified. Preparation of additional monoclonal antibodies, protein information, and RNA analysis, is underway. This information will provide the necessary base for the preparation of vaccines based on neutralizing, but non-toxic, recombinant virus-like proteins.

Until such "designed" vaccines become available, any of the isolated virus preparations each given the designation GLS<sub>n</sub> (n=1-116 currently) can be used, in killed form, for the preparation of conventional killed vaccines, which do confer immunity against the new virus. The GLS strains may be prepared into a vaccine through common methods, which are not per se a part of this invention among the most prominent of which are heat killing and chemical killing, which preserves the essential form of the vaccine to enable the preparation, by the inoculated bird, of protective NV antibodies while rendering it non-virulent. Alternatively, there are known methods of attenuating viruses, including serial passage, cloning of the virus deleting sequences of nucleic acids and site-directed mutagenesis, which will allow the preparation of a live non-virulent virus vaccine. The vaccines may be prepared by simple incorporation of the selected virus derivative and suspending or mixing it in a carrier. Appropriate dosage values can be determined through routine trial and error techniques, sampling for antibody titer.

As important as the preparation of the new vaccine is, there is now provided a method by which the presence of the virus can be identified in a given poultry population, by a relatively quick and efficient ELISA assay, which, if reaction to R63 alone, or R63 and B69 together is neg-

ative, while the reaction to a polyclonal vaccine or B29, is positive, then the presence of the virus is confirmed. B29 is expressed by a hybridomal cell line which has been deposited, under Budapest Treaty terms at the ATCC, under accession number HB 9746.

As of the filing of this application, at least 333 flocks had been so tested. Of these, 116 tested negatively against the R63 monoclonal antibody alone, or R63 and B69, taken together while positively for the B29 MCA. Thus, the new virus appears to be a dominant and growing factor in the causation of infectious bursal disease in chickens.

It is uncertain, as of the filing date of this application, whether the newly identified virus is a subtype or constitutes a new IBDV serotype, although there is considerable evidence that it is a serotype One subtype. In any event, identification of the presence of the virus and the preparation of a vaccine therefrom is achieved in the same manner whether strain or serotype, and accordingly, the invention is not limited thereby.

#### Claims

1. A purified preparation of virus capable of inducing infectious bursal disease in poultry, to which monoclonal antibody R63 (ATCC HB-9437) will not bind.
2. A purified preparation according to claim 1 to which a non-neutralizing test factor comprising infectious bursal disease antibodies will bind.
3. A preparation according to claim 2, wherein said test factor is selected from the group consisting of polyclonal antisera USDA ADV 8701 and monoclonal antibody B29 (ATCC MB 9746).
4. A preparation according to any preceding claim, wherein said virus is suspended in either a non-immunogenic medium or an immunogenic medium.
5. A preparation according to any preceding claim, wherein monoclonal antibody B69 (ATCC HB 9490) will not bind to said virus.
6. A preparation according to any preceding claim, which is deposited under accession number i-792 or i-793.
7. A vaccine for the prevention of infectious bursal disease in poultry, comprising:
  - a virus capable of inducing infectious bursal disease in poultry, to which virus monoclonal antibody R63 (ATCC HB-9437) will not bind, and a pharmacologically acceptable carrier therefore.
8. A vaccine according to claim 7, wherein said virus is in a killed form.
9. A vaccine according to claim 7, wherein said virus is in a live but attenuated form.
10. A vaccine according to any of claims 7 to 9, further comprising monoclonal antibody R63.
11. A vaccine according to any of claims 7 to 10, further comprising monoclonal antibody B69 (ATCC HB 9490).
12. A test kit for detecting the presence of a virus capable of inducing infectious bursal disease in a poultry population, comprising:
  - a preparation of monoclonal antibody R63 (ATCC HB-9437),
  - a preparation of a non-neutralizing test factor comprising antibodies to infectious bursal disease, and
  - means for supporting said preparations in contact with a sample of bursal tissue drawn from said population.
13. A kit according to claim 12, further comprising a preparation of monoclonal antibody B69 (ATCC HB 9490).
14. A kit according to claims 12 or 13, wherein said test factor comprises antibodies selected from the group consisting of monoclonal antibody B29 (ATCC MB 9746) and the antibodies present in polyclonal antiserum USDA ADV 8701.
15. A method for detecting the presence of a virus capable of inducing infectious bursal disease in a poultry population, which virus is not neutralized by a monoclonal antibody R63 (ATCC HB-9437), comprising:
  - contacting samples of bursal tissue separately with monoclonal antibody R63 and a positive non-neutralizing test factor capable of binding to said virus, and comparing the degree of binding observed between said samples and said monoclonal antibody and test factor,
  - wherein a high degree of binding between said sample and said test factor together with a low degree of binding between R63 and said sample is indicative of the presence of said virus.
16. A method according to claim 15, wherein said test factor comprises antibodies selected from the group consisting of monoclonal antibody B29 (ATCC MB 9746) and the antibodies present in polyclonal antiserum USDA ADV 8701.
17. A method according to claims 15 or 16, further comprising contacting said samples with monoclonal antibody B69 ATCC HB 9490, wherein a low degree of binding therebetween is further indicative of the presence of said virus.

18. A method for detecting the presence of a virus capable of inducing infectious bursal disease in a poultry population, which virus is not neutralized by monoclonal antibody R63 (ATCC HB-9437), comprising:  
 5 detecting the presence of an infectious bursal disease virus in said population by morphological or pathological inspection, and  
 contacting samples of bursal tissue drawn from said population with monoclonal antibody R63, and observing the degree of binding therebetween,  
 10 wherein a low degree of binding therebetween indicates the presence of said virus.
19. A purified preparation of virus according to any one of claims 1 to 6, for use in the preparation of a vaccine for the treatment of infectious bursal disease in poultry.

#### Patentansprüche

1. Gereinigte Viruspräparation, die zur Induzierung einer infektiösen Erkrankung der Bursa Fabricii in Geflügel in der Lage ist, an die monoklonale Antikörper R63 (ATCC HB-9437) nicht binden.
2. Gereinigte Präparation gemäß Anspruch 1, an die ein nicht-neutralisierender Testfaktor, umfassend Antikörper gegen die infektiöse Erkrankung der Bursa Fabricii, bindet.
3. Präparation gemäß Anspruch 2, wobei der Testfaktor aus der aus polyklonalem Antiserum USDA ADV 8701 und monoklonalem Antikörper B29 (ATCC MB 9746) bestehenden Gruppe gewählt wird.
4. Präparation gemäß einem der vorhergehenden Ansprüche, wobei der Virus entweder in einem nicht-immunisierenden oder in einem immunisierenden Medium suspendiert wird.
5. Präparation gemäß einem der vorhergehenden Ansprüche, worin der monoklonale Antikörper B69 (ATCC HB 9490) nicht am Virus bindet.
6. Präparation gemäß einem der vorhergehenden Ansprüche, welche unter der Zugangsnummer i-792 oder i-793 hinterlegt ist.
7. Impfstoff zur Verhinderung einer infektiösen Erkrankung der Bursa Fabricii in Geflügel, umfassend:  
 50 ein Virus, das zur Induzierung einer infektiösen Erkrankung der Bursa Fabricii bei Geflügel in der Lage ist, wobei der monoklonale Antikörper R63 (ATCC HB-9437) nicht an dem Virus bindet, und einen pharmakologisch annehmbaren Träger dafür.
8. Impfstoff gemäß Anspruch 7, worin der Virus in abgetöteter Form vorliegt.
9. Impfstoff gemäß Anspruch 7, worin der Virus in lebender aber abgeschwächter Form vorliegt.
10. Impfstoff gemäß einem der Ansprüche 7 bis 9, weiterhin den monoklonalen Antikörper R63 umfassend.
11. Impfstoff gemäß einem der Ansprüche 7 bis 10, weiterhin den monoklonalen Antikörper B69 (ATCC HB 9490) umfassend.
12. Testkit für den Nachweis der Anwesenheit eines Virus, der zur Induzierung einer infektiösen Erkrankung der Bursa Fabricii in einer Geflügelpopulation in der Lage ist, umfassend:  
 eine Präparation monoklonaler Antikörper R63 (ATCC HB-9437),  
 eine Präparation eines nicht-neutralisierenden Testfaktors, umfassend Antikörper gegen die infektiöse Erkrankung der Bursa Fabricii, und  
 20 Mittel zum Unterstützen dieser Präparationen beim Kontakt mit einer Probe von Bursa Fabricii-Gewebe aus der Population.
13. Kit gemäß Anspruch 12, weiterhin eine Präparation monoklonaler Antikörper B69 (ATCC HB 9490) umfassend.
14. Kit gemäß Anspruch 12 oder 13, wobei der Testfaktor aus der aus monoklonalem Antikörper B29 (ATCC MB 9746) und den in dem polyklonalen Antiserum USDA ADV 8701 vorliegenden Antikörpern bestehenden Gruppe gewählte Antikörper umfaßt.
15. Verfahren zum Nachweis der Anwesenheit eines Virus, das zur Induzierung einer infektiösen Erkrankung der Bursa Fabricii in einer Geflügelpopulation in der Lage ist, wobei der Virus nicht durch einen monoklonale Antikörpern R63 (ATCC HB-9437) neutralisiert ist, umfassend:  
 40 Kontaktieren von Proben des Bursa Fabricii-Gewebes getrennt mit monoklonalem Antikörper R63 und einem positiven nicht-neutralisierenden Testfaktor, der zur Bindung an das Virus in der Lage ist, und Vergleichen des zwischen den Proben und dem monoklonalen Antikörper und Testfaktor festgestellten Bindungsgrades,  
 wobei ein hoher Bindungsgrad zwischen der Probe und dem Testfaktor zusammen mit einem niedrigen Bindungsgrad zwischen R63 und der Probe die Anwesenheit des Virus andeutet.
16. Verfahren gemäß Anspruch 15, wobei der Testfaktor Antikörper umfaßt, die aus der aus dem monoklonalen Antikörper B29 (ATCC MB 9746) und den in dem polyklonalen Antiserum USDA ADV 8701 vorliegenden Antikörpern bestehenden Gruppe gewählt werden.

17. Verfahren gemäß Anspruch 15 oder 16, das weiterhin das Kontaktieren der Proben mit monoklonalem Antikörper B69 (ATCC HB 9490) umfaßt, wobei ein niedriger Bindungsgrad zwischen ihnen weitergehend die Gegenwart des Virus andeutet.

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18. Verfahren zum Nachweis der Anwesenheit eines Virus, der zur Induzierung einer infektiösen Erkrankung der Bursa Fabricii in einer Geflügelpopulation in der Lage ist, wobei der Virus nicht durch den monoklonalen Antikörper R63 (ATCC HB-9437) neutralisiert ist, umfassend:

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Nachweisen des Vorhandenseins eines die infektiöse Erkrankung der Bursa Fabricii in der Population auslösenden Virus durch morphologische oder pathologische Inspektion, und Kontaktieren von Proben des von der Population gezogenen Bursa Fabricii-Gewebes mit dem monoklonalen Antikörper R63 und Feststellen des zwischen ihnen vorliegenden Bindungsgrades, wobei ein niedriger Bindungsgrad die Anwesenheit des Virus andeutet.

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19. Gereinigte Viruspräparation gemäß einem der Ansprüche 1 bis 6 zur Verwendung bei der Herstellung eines Impfstoffes zur Behandlung der infektiösen Erkrankung der Bursa Fabricii in Geflügel.

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#### Revendications

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1. Préparation purifiée de virus capable d'induire une bursite infectieuse chez la volaille, à laquelle l'anticorps monoclonal R63 (ATCC HB-9437) ne se lie pas.

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2. Préparation purifiée selon la revendication 1 à laquelle un facteur de test non neutralisant comprenant des anticorps de bursite infectieuse se lie.

3. Préparation selon la revendication 2, dans laquelle ledit facteur de test est choisi parmi le groupe consistant en antisérums polyclonaux USDA ADV 8701 et l'anticorps monoclonal B29 (ATCC MB 9746).

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4. Préparation selon l'une quelconque des revendications précédentes, dans laquelle ledit virus est mis en suspension soit dans un milieu non immunogène soit dans un milieu immunogène.

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5. Préparation selon l'une quelconque des revendications précédentes, dans laquelle l'anticorps monoclonal B69 (ATCC HB 9490) ne se lie pas audit virus.

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6. Préparation selon l'une quelconque des revendications précédentes, qui a été déposée sous le numéro de dépôt i-792 ou i-793.

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7. Vaccin pour la prévention de bursite infectieuse chez la volaille, comprenant :

un virus capable d'induire une bursite infectieuse chez la volaille, virus auquel l'anticorps monoclonal R63 (ATCC HB-9437) ne se lie pas, et un véhicule pharmaceutiquement acceptable de celui-ci.

8. Vaccin selon la revendication 7, dans lequel ledit virus est sous une forme tuée.

9. Vaccin selon la revendication 7, dans lequel ledit virus est sous une forme vivante mais atténuée.

10. Vaccin selon l'une quelconque des revendications 7 à 9, comprenant en outre l'anticorps monoclonal R63.

11. Vaccin selon l'une quelconque des revendications 7 à 10, comprenant en outre l'anticorps monoclonal B69 (ATCC HB 9490).

12. Kit de test pour détecter la présence d'un virus capable d'induire une bursite infectieuse dans une population de volaille, comprenant :

une préparation d'anticorps monoclonal R63 (ATCC HB-9437),

une préparation d'un facteur de test non neutralisant comprenant des anticorps dirigés contre la bursite infectieuse, et

un moyen pour maintenir en contact lesdites préparations avec un échantillon de tissu de bourse prélevé à partir de ladite population.

13. Kit selon la revendication 12, comprenant en outre une préparation d'anticorps monoclonal B69 (ATCC HB 9490).

14. Kit selon les revendications 12 ou 13, dans lequel ledit facteur de test comprend des anticorps choisis parmi le groupe consistant en l'anticorps monoclonal B29 (ATCC MB 9746) et les anticorps présents dans l'antisérum polyclonal USDA ADV 8701.

15. Méthode pour détecter la présence d'un virus capable d'induire une bursite infectieuse dans une population de volaille, virus qui n'est pas neutralisé par un anticorps monoclonal R63 (ATCC HB-9437), comprenant les étapes consistant à :

mettre en contact des échantillons de tissu de bourse séparément avec l'anticorps monoclonal R63 et un facteur de test non neutralisant positif capable de se lier audit virus, et comparer le degré de liaison observé entre lesdits échantillons et lesdits anticorps monoclonal et facteur de test,

où un degré élevé de liaison entre ledit échantillon et ledit facteur de test accompagné d'un faible degré de liaison entre R63 et ledit échantillon indique la présence dudit virus.

16. Méthode selon la revendication 15, dans laquelle ledit facteur de test comprend des anticorps choisis parmi le groupe consistant en l'anticorps monoclonal B29 (ATCC MB 9746) et les anticorps présents dans l'antisérum polyclonal USDA ADV 8701. 5
17. Méthode selon les revendications 15 ou 16, comprenant en outre une étape consistant à mettre en contact lesdits échantillons avec l'anticorps monoclonal B69 (ATCC HB 9490), où un faible degré de liaison 10 entre ceux-ci indique en outre la présence dudit virus.
18. Méthode pour détecter la présence d'un virus capable d'induire une bursite infectieuse dans une population de volaille, virus qui n'est pas neutralisé par l'anticorps monoclonal R63 (ATCC HB-9437), comprenant les étapes consistant à : 15
- détecter la présence d'un virus de bursite infectieuse dans ladite population par un examen morphologique ou pathologique, et 20
- mettre en contact les échantillons de tissu de bourse prélevés à partir de ladite population avec l'anticorps monoclonal R63, et observer le degré de liaison entre ceux-ci, 25
- où un faible degré de liaison entre ceux-ci indique la présence dudit virus.
19. Préparation purifiée de virus selon l'une quelconque des revendications 1 à 6, utilisable dans la préparation d'un vaccin pour le traitement de bursite infectieuse chez la volaille. 30

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